

End of Result Set

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L6: Entry 1 of 1

File: USPT

Jan 21, 1997

DOCUMENT-IDENTIFIER: US 5595890 A

TITLE: Method of detecting nucleotide sequences

Brief Summary Text (55):

If only 1, 2 or 3 nucleoside triphosphates are present then the diagnostic primer will only extend as far as the presence of these nucleoside triphosphates will permit. As indicated above, where there is a mismatch between for example the 3' terminal end of the diagnostic primer and the corresponding nucleoside triphosphate in the sample nucleic acid no primer extension will be effected. Where, however, the 3' terminal nucleoside triphosphate is complementary with the corresponding nucleoside triphosphate in the sample nucleic acid, primer extensions will be effected.

Brief Summary Text (79):

It will be appreciated, however, that in certain circumstances synthesis of a diagnostic primer extension product might be induced to occur even in the presence of a non-complementary 3'-terminal residue. This artefactual result may arise from the use of too low a temperature in which case the temperature may be increased, too long a time of incubation/annealing in which case the time may be reduced, too high a salt concentration in which case the salt concentration may be reduced, too high an enzyme concentration, too high a nucleoside triphosphate concentration, an incorrect pH or an incorrect length of oligonucleotide primer. All of these factors are discussed in European Patent Publication No 237,362. A major source of artefactual products is probably allowing the reaction temperature to fall too low, thus permitting too low a stringency, for example by removing the reaction mixture from the heat cycling means, even briefly for example to add the agent for polymerisation (eg. Taq polymerase) especially in the first reaction cycle. In addition to the above we have found that such artefactual results may also arise from use of a diagnostic primer which is particularly rich in G (guanosine) and C (cytidine) residues. A diagnostic primer may give rise to difficulty in this regard if it is G/C rich as a whole or particularly if it is G/C rich at its relevant, normally 3', end. Moreover the precise nature of the base pairing in the region of the relevant, normally 3', end of the diagnostic primer when in use may be the cause of an artefactual result. Thus the presence of As (adenosine) in the base pairing in the region of the relevant, normally 3', end of the diagnostic primer tends to improve specificity whilst the presence of Gs (guanosine) does not. Furthermore the precise nature of the mismatch at the relevant, normally 3', end of the diagnostic primer may be an important factor in whether or not an artefactual result is obtained. Thus for example an AA or CT mismatch does not normally result in hybridisation, but a GT or AC mismatch may result in a sufficient degree of hybridisation to result in the formation of artefactual product(s). Artefactual results may be avoided by deliberately introducing one or more further mismatched residues, or if desired, deletions or insertions, within the diagnostic primer to destabilise the primer by further reducing the binding during hybridisation.

Brief Summary Text (80):

Thus for example any one or more of the 10, for example 6 nucleotides adjacent to the terminal mismatch may be altered to introduce further mismatching. In general only one mismatch in addition to the terminal mismatch may be necessary, positioned for example, 1, 2 or 3 bases from the terminal mismatch. Thus, for example, in relation to the determination of the presence of a normal homozygote, heterozygote or affected homozygote in respect of the Z allele of the .alpha.1 antitrypsin gene we have found that good results may be obtained if the third nucleotide from the 3' terminal nucleotide is altered to generate a mismatch in use. Thus for example we have found that the presence of a C instead of an A as the third nucleotide from the 3' terminus

of the diagnostic primer enables normal homozygotes, heterozygotes and affected homozygotes in respect of the Z allele to be readily distinguished. The best design of diagnostic primer may thus be determined by straightforward experimentation based on the above criteria, such experimentation being well within the ability of the skilled molecular biologist.

Detailed Description Text (59):

Example 2 thus demonstrates that a mismatch at the 3' end of an oligonucleotide primer prevents or at least substantially inhibits the initiation of polymerase activity.

Other Reference Publication (29):

Saiki et al, "Analysis of enzymatically amplified .beta.-globin and HLA-DQ.alpha. DNA with allele-specific oligonucleotide probes", Nature 324:163-166 (1986).